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RESEARCH ARTICLE

Reproductive ecology and territorial behavior of *Boana goiana* (Anura: Hylidae), a gladiator frog from the Brazilian Cerrado

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ABSTRACT. Anuran males and females adopt different reproductive and behavioral strategies in different contexts. We investigated the reproductive ecology and territorial behavior of the treefrog *Boana goiana* (B. Lutz, 1968) from the Brazilian Cerrado. We hypothesized that competitor density/proximity would increase the behavioral responses of *B. goiana* males, and that mating would be assortative. We also tested if the number of eggs correlates with female size and if there is a trade-off between clutch size and egg size. We conducted two territoriality experiments to test the effects of male size, competitor proximity and competitor density. Larger males called more in the presence of a second male. In the second experiment, the largest males emitted more calls and the distance to the nearest male increased as resident males called more. In both experiments, the number of calls was influenced by either male size or spacing between males. Some males behaved as satellites, probably to avoid fights. Our analyses indicate that females choose males with similar sizes to their own, corroborating our hypothesis of size-assortative mating. We found no relationships between female size and clutch size/volume, and between egg size and number of eggs per clutch. We also report multiple spawning for this species. The low incidence of physical combats and the spacing pattern indicate that this species relies almost solely on calls to resolve contests, which could be explained by low motivation, or simply because males avoid combats to decrease injury risks. Thus, acoustic or even multimodal communication seems crucial for social interactions of *B. goiana*.

KEY WORDS. Clutch size, acoustic communication, assortative mating, male competition, aggressive interactions, arrival dynamics.

INTRODUCTION

In the classic Darwinian definition of sexual selection, in animals that reproduce sexually, males and females will adopt different strategies to ensure their reproductive success (Andersson 1994, Candolin 2019). Because their reproduction is limited in time and space (Haddad and Prado 2005, Wells 2007), anuran amphibians are good model organisms to investigate factors related to mating and reproductive success, with males and females adopting markedly different strategies (Cayuela et al. 2017, Valencia-Aguilar et al. 2020). In general terms, the reproductive success of males of many animal species can be defined by a variety of factors, including body size, territory quality, displays, and built structures (e.g. fish: McKaye et al. 1990, frogs: Olsson and Shine 1996, crabs: Murai and Backwell 2006). Specifically for male anurans, arrival at the reproductive area is often associated with mating success, and males that arrive early may increase their chances of mating (Lodé et al. 2005, Dias et al. 2017). The establishment and maintenance of a territory can also be essential for the reproductive success of anuran males (Wells 1977a). Regarding the ability to establish and maintain territories by males, acoustic signaling is an essential component of anuran social behavior (Wells 2007). Male anurans use calls to establish territories, interact aggressively with other males, maintain the spacing among calling neighbors and – most importantly – to attract females (Bastos

et al. 2011, Lemes et al. 2012). The calling activity of neighbors may influence the resident males' behavior, changing their acoustic behavior and sometimes leading to physical combats (Wells 1977a, Bastos et al. 2011, Lemes et al. 2012). Males can also assess the size of rival males by evaluating call parameters, thus avoiding a conflict with a larger opponent (Bee and Perrill 1996, Bee et al. 1999, Nali and Prado 2014). Furthermore, the establishment of a territory by one of the males can affect the outcome of the contest by increasing motivation to defend its territory (Owen and Gordon 2005).

Meanwhile, the reproductive success of females is usually affected by mate choice and their capacity to produce oocytes, which generally correlates with body size (e.g. lizards and snakes: Shine 1992, frogs: Pettitt et al. 2013). In anurans, the reproductive effort of females usually fits one of three patterns: clutches with a few large eggs, clutches with many small eggs, or variable egg sizes within the same clutch (Crump 1984, Dziminski et al. 2009, Pupin et al. 2018). Additionally, large females can produce more eggs than small females, and this positive relationship between clutch size and female size is known for several anuran species (e.g. Kusano and Hayashi 2002, Wogel et al. 2002, Dziminski and Alford 2005). In this context, the reproductive strategies of females may vary depending on the environmental conditions (Crump 1984, Pupin et al. 2018). Another important aspect is mate choice and anuran females often choose their mates (Morris 1989), favoring males that may confer them the greatest reproductive success, but strategies may vary among species (Pfennig 2000, Pettitt et al. 2013, Valencia-Aguilar et al. 2020). Within the broader framework of sexual selection, some species may exhibit assortative mating. In such cases, females may choose males with traits similar to their own, such as those with aposematic color patterns (Reynolds and Fitzpatrick 2007) or males of similar body sizes, which allows for a closer juxtaposition of the cloacae during mating, increasing fertilization rates (Licht 1976, Bastos and Haddad 1996).

Although anuran territorial behavior is well studied (Fellers 1979, Bee 2016), the vast majority of the knowledge regarding this issue comes from direct natural history observations (e.g. Oliveira et al. 2011) or experiments using playbacks (e.g. Amézquita et al. 2005). Regardless of being well established in herpetology, these approaches have some limitations: naturalistic observations depend on chance to describe some behaviors and are purely descriptive, while playback experiments are unable to address multimodal communication. In contrast, in situ experiments investigating interactions between two or more individuals are rare (e.g. Hagen et al. 2016), however, they allow for a factorial design and can be more informative than the aforementioned approaches. This becomes more important when we consider that multimodal signaling seems to be more common and important for anuran social behavior than previously thought (Hartmann et al. 2004, Richardson et al. 2010, Starnberger et al. 2014).

Boana goiana (B. Lutz, 1968) is an interesting model organism to study aspects of the reproductive biology of both male and female anurans. The species is a gladiator frog distributed in the central Brazilian states of Goiás, Minas Gerais and in the Distrito Federal (Frost 2020). In the rainy season, males of this species call perched on the vegetation surrounding water bodies (Menin et al. 2004). Furthermore, males of *B. goiana* are known to display territorial behavior and to be faithful to calling sites (Menin et al. 2004, Dias et al. 2017). Although some studies on the species' biology have already been published (Bastos et al. 2003, Menin et al. 2004, Morais et al. 2016, Dias et al. 2017), little is known about the reproductive ecology and dynamics of male territoriality. Because of their territorial behavior and the presence of weaponry in males, *B. goiana* is particularly suitable to investigate the effect of competitor density on the reproductive and territorial behavior of hylids. Competitor density is known to affect territorial and reproductive behavior in many animal groups, such as teleost fishes (e.g. Kaspersson et al. 2010, Magellan et al. 2011), spiders (e.g. Ritschoff 2010) and passerine birds (e.g. Foltz et al. 2015). Investigations regarding the effect of competitor density in the social behavior of amphibians, conversely, focus mostly on issues such as mating success and amplexus duration (e.g. Chuang et al. 2013), with territoriality being a neglected aspect. The concept of economic defendability predicts that male competition will increase with density if the resource value outweighs the cost of defense (Grant 1993). This prediction can be applied to *B. goiana*: mating is restricted to the reproductive season, meaning that female availability is restricted in time, and males occupy and defend spatially limited calling perches (Dias et al. 2017). In this context, competitor density and prior establishment of territories, i.e. the resident effect (Baugh and Forester 1994), likely affect the social behavior of *B. goiana*. Lastly, studies that provide basic information of a species' life history, ecology and behavior are important because not only they provide basic information for conservation purposes, but they also provide background knowledge for testing more elaborated hypotheses, and *B. goiana* is a potentially interesting organism to provide such information.

In the present study, we investigated the reproductive ecology and the dynamics of the territorial behavior of *B. goiana*, combining in-situ experiments with natural history observations. We hypothesized that: 1) because size is related to success in contests, large males will be more aggressive and will dominate interactions without a prior resident, and these contests will escalate with time; 2) because resources are restricted across time and space, an increase in competitor density will increase the intensity of aggressive behaviors displayed by resident males towards intruders; and 3) because similar body sizes facilitate cloacal juxtaposition, females will display size-assortative mate choice. We also tried to answer the following questions: a) Do large males arrive at the reproductive area early in the reproductive season? b) Is there a positive relationship between female size and clutch size? c) Is there a trade-off between egg size and number of eggs per clutch?

MATERIAL AND METHODS

The study was conducted at the Floresta Nacional de Silvânia (FNS; 16°39'32"S, 48°36'29" W, elevation ca. 900 m), municipality of Silvânia, state of Goiás, central-western Brazil. The FNS is located in the Cerrado domain and includes many vegetation types, one of which is the gallery forest, where we conducted this study (sensu Oliveira-Filho and Ratter 2002). According to Koppen's classification (Peel et al. 2007), the study area is characterized by a tropical savanna climate (Aw), with two well-defined seasons, a rainy season from October to March and a dry season from April to September.

Data collection

Territoriality

During the reproductive seasons of 2012–13 and 2013–14, we conducted territoriality experiments in the field. We placed *B. goiana* males 100 cm above the ground because this height is the most commonly observed in nature (Menin et al. 2004). The usual distance between males in natural environments is around 200 cm, and distances smaller than 100 cm are rare (Menin et al. 2004). Thus, we placed males 50 cm apart to maximize their behavioral response in the establishment and defense of the territory.

To test our first two hypotheses, we designed two territoriality experiments. In the first experiment we tested if male size related to calling behavior and social status in a contest without prior residence – since resident males may be more likely to win due to different motivation rates (Baugh and Forester 1994). For that, we chose two males in calling activity that were at approximately 2 m from each other. Next, the two males were taken to another place within the reproductive area. There, the males were placed on a small tree 100 cm above the ground and were initially 50 cm apart. We waited until one of them started calling and counted the number of advertisement calls and aggressive calls emitted by both males during two minutes. The counting of the calls was repeated 15 and 30 minutes later, totaling three counting periods (initial, 15, 30 min). We also determined the distance between the males with a measuring tape in each counting period. After the third counting period, the experiment was terminated and males were measured with a digital caliper (0.01 mm). We replicated this experiment 15 times with different males at each trial. For each two-minute counting period performed, the behavioral responses of males were also recorded using the focal animal method (Altman 1974).

To test our second hypothesis, we designed a second experiment, in which we arrived at the reproductive area and searched for two males that were up to 400 cm apart, so that we could see both simultaneously. These males were considered resident males. During two minutes we counted the number of advertisement calls and aggressive calls emitted by them. Following this, we increased male density by searching for a third male (first intruder), which was placed between the resident males. We waited until the intruder male started to call and once again counted the number of advertisement and aggressive calls emitted by the resident males, during two minutes. We also used a measuring tape to measure the distance between all males. This procedure was repeated until a second and then a third intruder was placed in the area. After counting the calls and measuring the distance between the males, the experiment was terminated. A total of four counting periods were performed, including: 1) two resident males; 2) adding one intruder male; 3) adding a second intruder; 4) adding a third intruder. We replicated this experiment six times, with different males used at each trial. Males were also measured as described above. For each two-minute counting period performed, the behavioral responses of males were also recorded employing the focal animal method (Altman 1974).

Reproductive ecology

We visited the reproductive area during the 1996–97, 1997–98, 2005–06, 2006–07, 2012–13 and 2013–14 rainy seasons. Data obtained before 2012 were used only to verify size-assortative mating and test our third hypothesis. Data for the remaining exploratory objectives were obtained exclusively from 2012 onwards. We searched for pairs in amplexus after sunset (around 07:00 p.m.) and, when captured, placed them in plastic bags containing water and waited for them to spawn; after which we returned the pair and the clutch to the sample site. Amplectant pairs were collected because the reproductive site (a stream) used by *B. goiana* prevented us from sampling eggs spawned directly in the field – some eggs could be carried away by the water flow and/or remain hidden in detritus, resulting in unreliable data. For each clutch we counted the number of eggs and collected 10 eggs to be measured. The eggs were fixed in 10% formalin and deposited at the Coleção Zoológica da Universidade Federal de Goiás (ZUFG). We measured egg diameter in the laboratory using a Zeiss stereomicroscope with a scaled background, to the nearest 0.01 mm, and calculated clutch volume according to Guimarães et al. (2011). We also measured the snout-vent length (SVL) and mass of all males and females with a digital caliper (0.01 mm) and a digital scale (0.01 g), respectively. Lastly, we also marked the individuals with subcutaneous implants (alphanumeric fluorescent tags visible under ultraviolet light, Northwest Marine Technology, Inc.) to avoid pseudoreplication.

Data analyses

Before performing the analyses, all variables were log-transformed and tested for normal distribution (Zar 1999). A ln(x+1) transformation was adopted if the data matrix included zeroes, whilst a ln(x) transformation was adopted for the remaining variables. All tests were performed in the R software (R Development Core Team 2013).

Territoriality

For each replicate of the first experiment, we labelled the male who emitted more calls as the alpha, and the other was considered the beta. To determine the establishment of dominance between the males and the validity of our alpha-beta

classification, we tested if the supposed alphas and betas differed in the number of calls emitted during the experiment using a paired t-test. Since the difference was statistically significant ($t_{[14]} = 6.35$, $p < 0.001$), i.e., alpha males emitted more calls, we considered our classification as valid.

To test whether alpha males were larger than betas, we compared their SVL with a paired t-test. For the experiments, we considered the variable "male size" as a composite measure obtained by using the scaled mass index, an allometric index that accounts for the different scaling rates of body length and mass (Peig and Green 2009). To investigate if male behavior escalated through time, we tested whether number of calls emitted by males varied among the counting periods (initial, 15 min, and 30 min later) performing two repeated-measure ANCOVAs (one test for the advertisement calls and another for the aggressive calls). We used the distance between males and male size as covariates. For these tests, only one randomly chosen male (either the alpha or the beta male) was included in the analyses. Thus, while there was only one distance value possible, the variable "male size" always corresponded to the male that was chosen.

In the second experiment, to evaluate if an increase in the number of nearby males affected the number of calls emitted by the resident males, we compared these variables among the counting periods. For that, we performed two repeated-measure ANCOVAs (one test for the advertisement calls and another for the aggressive calls), using the distance to the nearest male and male size as covariates. The variable "male size" was determined as described for the first experiment. For these tests, only one randomly chosen resident male was included in the analyses. Thus, both "distance to the nearest male" and "male size" referred specifically to the male that was chosen.

Reproductive ecology

To analyze if mating was assortative regarding body size, we performed a Pearson's Correlation test between the SVLs of males and females in amplexus. To increase the robustness of this analysis, we included previously obtained data from the 1996–97, 1997–98, 2005–06 and 2006–07 reproductive seasons, as well as the data obtained from 2012–2013 onwards. To examine if clutch size (number of eggs per clutch) and clutch volume (number of eggs per clutch*egg volume) were correlated with female SVL, and whether egg size was correlated with the number of eggs per clutch, we performed simple linear regressions. To examine the body size profile (and, therefore, the age) of males of the studied population, we performed a one-way ANOVA to test if SVL of males differed among the months of the reproductive season.

Ethical and legal standards

We performed the procedures and experiments detailed in this study in strict accordance to the Brazilian federal and state laws regulating the sampling of animal species, their welfare and their use for research purposes. Sampling and handling of animals were authorized under ICMBio permit #40668–1.

RESULTS

Territoriality

In the first experiment, alpha males were significantly larger than beta males (SVL, $t_{[14]} = 2.37$, $p = 0.03$) (Fig. 1). The SVL of alpha males ranged from 31.03 to 34.33 mm (average = 33.10 ± 0.94, n = 15) and body mass from 1.14 to 1.61 g (average = 1.41 ± 0.13, n = 15); while the SVL of beta males varied from 28.80 to 33.60 mm (average = 31.94 ± 1.42, n = 15) and body mass from 1.01 to 1.59 g (average = 1.33 ± 0.15, n = 15). The number of advertisement and aggressive calls emitted by males did not significantly differ among periods (i.e. behaviors did not escalate), and the number of advertisement calls was positively influenced by male body size, while the number of aggressive calls was positively influenced by the distance between males (Table 1).

Of a total of 15 replicates in the first experiment, the frequencies of the observed behavioral responses were as follows: (a) males only walked away from each other (n = 3); (b) males only approached each other (n = 2); (c) males walked away and then approached each other (n = 2); (d) males approached and then walked away from each other (n = 6); (e) one male amplected a female that approached him during the experiment (n = 1); (f) one male approached the other, initiated physical contact and caused both males to fall to the ground (n = 1).

In the second experiment, the number of aggressive calls emitted by resident males increased with male size and with the distance between males (Table 1). The number of advertisement calls was not influenced by any of the tested variables (Table 1). Frequencies of the observed behavioral responses, of a total of six replicates, were as follows: a) the introduced male behaved as

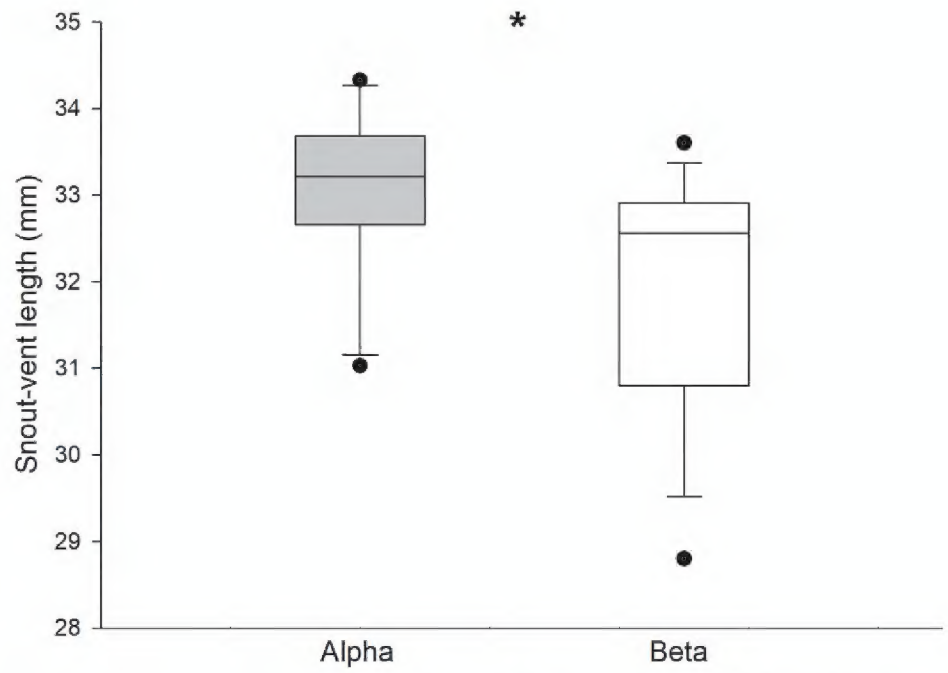


Figure 1. Body size (SVL) of the alpha and beta males of *Boana goiana* sampled in the first territoriality experiment, in Silvânia, Goiás, Brazil; bar height represents the range between the first and third quartiles, whiskers represent maximum and minimum values, horizontal bars represent the median, gray bar represents alpha males, white bar represents beta males, (*) indicates statistically significant differences.

Table 1. Results for the first and second territoriality experiments: the repeated measures ANCOVAs for the effect of the period, distance between the males and male size on the variation of the number of calls emitted by males of *Boana goiana* are shown for both experiments. Distance and size were used as covariates. Statistically significant values of "p" are shown in bold.

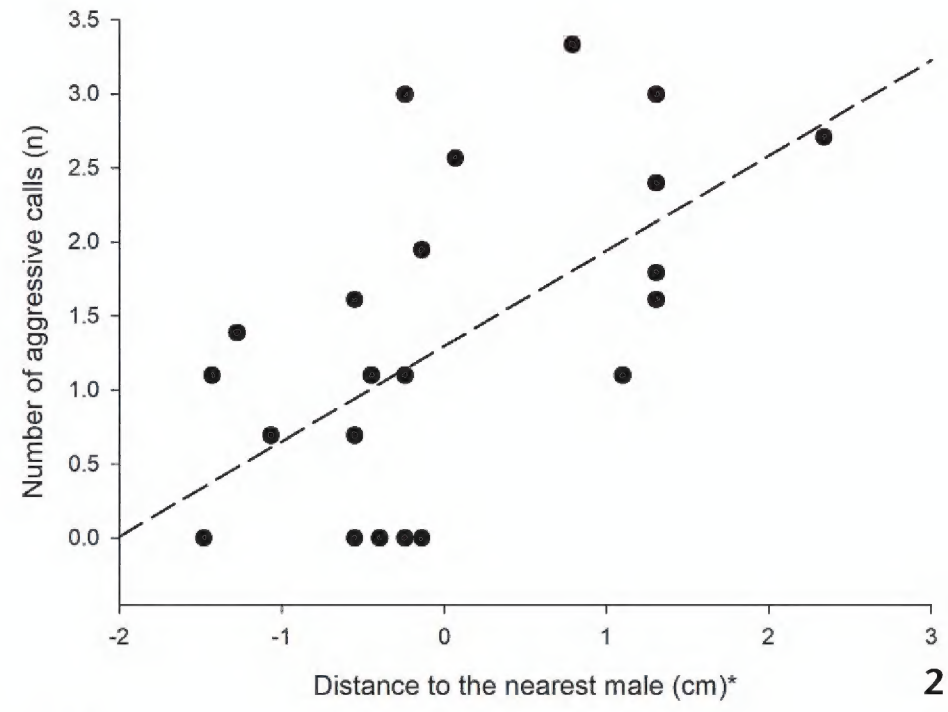
Call type/variable	Territoriality experiments					
	First			Second		
	df	F	p	df	F	p
Advertisement calls						
Period	2,34	1.526	0.22	3,17	0.93	0.45
Distance between males	1,34	1.123	0.29	1,17	0.136	0.71
Male size	1,34	14.913	< **0.001**	1,17	0.13	0.29
Aggressive calls						
Period	2,34	1.029	0.39	3,17	1.20	0.34
Distance between males	1,34	4.70	**0.03**	1,17	10.76	< **0.01**
Male size	1,34	1.77	0.19	1,17	9.81	< **0.01**

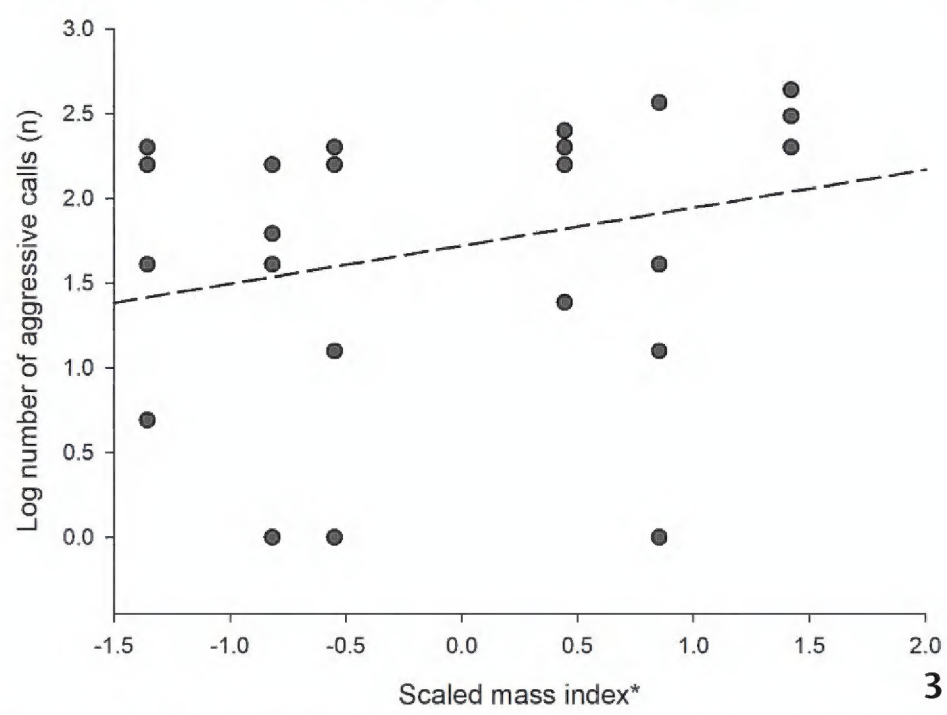
satellite male (n = 2); b) one resident male and one introduced male behaved as satellite males (n = 1); c) one resident male walked away (n = 1); d) the introduced male walked away (n = 2); e) one resident male engaged in physical combat with one introduced male, both fell to the ground and resumed calling activity after a few minutes (n = 1).

To further explore how the co-variables influenced calling behavior in the territoriality experiments, we ran a series of a posteriori tests. For the first experiment, we performed two random-effects GLMs, one for each type of call. We used a Poisson distribution model with a log link, and the repeated-measures (i.e. individuals across time) as the random effect. To fit these models, instead of an ln+1 transformation, we used raw data scaled within the model. The results showed that the number of advertisement calls was positively related to both male size and distance between the males, while the number of aggressive calls was positively related only to the distance between males (Table 2). The same approach was chosen for the second experiment, with one random-effect GLM for each type of call, using the same parameters described above. No effects were reported for the advertisement calls, while aggressive calls were positively influenced by both male size and distance to the nearest male (Table 2, Figs 2, 3). Because the ANCOVAs showed the effect of time to be non-significant (Table 1), we dismissed it as a variable in the GLMs. We performed these analyses in the R software using the "nlme4" package.

Reproductive ecology

The clutches contained on average 164.88 ± 30.18 eggs (range = 105 – 207 eggs; n = 9) and the average diameter of eggs was 1.68 ± 0.16 mm (range = 1.39 – 1.96 mm; n = 90 eggs from 9 clutches). As we marked and recaptured individuals (Table 3), we were able to observe that from the nine clutches we sampled, two were fertilized by the same male (176 and 150 eggs, with a 24 h interval between them) and two were laid by the same female (151 and 207 eggs, with a 33-day interval between them).





Figures 2–3. Relationship between the number of aggressive calls emitted by the resident male and (2) the distance to the nearest male, and (3) male size of *Boana goiana* in the second territoriality experiment, in Silvânia, Goiás, Brazil; (*) predictor variables were scaled to fit the model.

Male SVL ranged from 28.53 to 37.48 mm (average = 32.55 ± 1.46 mm, n = 217) and male mass from 1.01 to 1.61 g (average = 1.34 ± 0.13, n = 57). Female SVL ranged from 34.40 to 37.48 mm (average = 35.84 ± 0.10, n = 8) and female mass from 1.39 to 1.92 g (average = 1.72 ± 0.19, n = 8). We did not find any relationship between female SVL and the number of eggs per clutch, between female SVL and clutch volume, nor between egg size and the number of eggs per clutch ($F_{[1,7]} = 1.32$, $r^2_{adj} = 0.04$, $p = 0.28$; $F_{[1,7]} = 1.39$, $r^2_{adj} = 0.04$, $p = 0.27$; $F_{[1,7]} = 0.43$, $r^2_{adj} = -0.076$, $p = 0.53$; n = 8, respectively). Body size (SVL) of males and females in amplexus were positively correlated ($r = 0.65$, $p < 0.001$, n = 14). Male to female SVL ratio averaged 0.911 ± 0.03 (range = 0.86–0.97 mm). Male SVL varied among months, i.e., in the last months of the reproductive season (March, April and May), males were smaller compared to males of all other months

Table 2. Results of the random-effect GLMs performed as a posteriori analyses for the two territoriality experiments; number of advertisement and aggressive calls were used as response-variables; predictor variables were distance between males and male size for the first experiment, and distance to the nearest male and male size for the second experiment; statistically significant values of "p" are shown in bold.

Call type/variable	Estimate	z	p
First territoriality experiment			
Advertisement calls			
Intercept	1.2129	7.706	**<0.01**
Distance between males	0.5734	3.335	**<0.01**
Male size	1.8780	2.859	**<0.01**
Aggressive calls			
Intercept	-1.3704	-1.847	0.0648
Distance between males	0.3961	4.340	**< 0.01**
Male size	0.3304	0.518	0.6044
Second territoriality experiment			
Advertisement calls			
Intercept	1.7652	15.235	**<0.01**
Distance to the nearest male	0.1678	1.895	0.06
Male size	0.2050	1.775	0.07
Aggressive calls			
Intercept	1.0217	3.116	**<0.01**
Distance to the nearest male	0.4437	4.550	**< 0.01**
Male size	0.8761	2.746	**< 0.01**

($F_{[5,211]}$ = 4.59, p < 0.001). Mean SVL of males from December to February was 32.75 ± 1.57 mm (range = 28.58–37.48 mm; n = 107) and from March to May it was 32.34 ± 1.31 mm (range = 28.53–35.23 mm; n = 110) (Fig. 4).

In the 2012–13 reproductive season, we marked 108 individuals (4 females and 104 males), and in the reproductive season of 2013–14 we marked 128 individuals (5 females and 123 males). However, no individual marked in the first season was recaptured in the second reproductive season. The total number of individuals captured and recaptured is shown in Table 3. Recapture intervals ranged from 1 to 84 days.

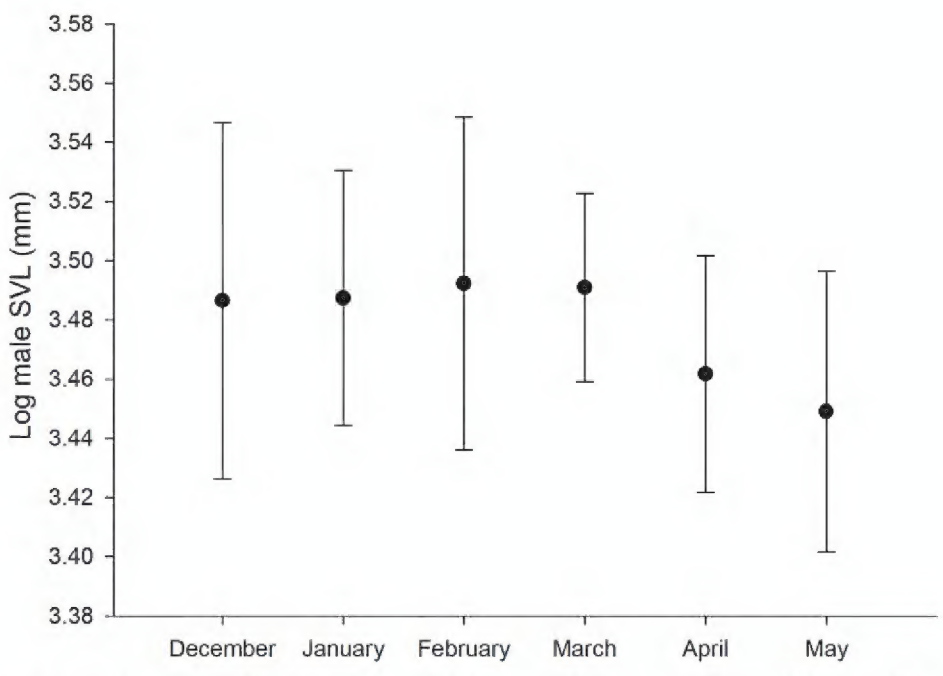


Figure 4. Body size (SVL) of males of *Boana goiana* along the months of the reproductive season 2013/2014, in Silvânia, Goiás, Brazil. Black circles indicate the means, whiskers indicate the standard deviation.

Table 3. Captures and recaptures of males of *Boana goiana* in Silvânia, Goiás, Brazil. First season: January 2013 to May 2013; Second season: October 2013 to May 2014.

Variable name	First season	Second season
Total of individuals captured	108	128
Total of recaptures	68	63
Recaptured one time	28	30
Recaptured two times	11	10
Recaptured three times	3	3
Recaptured four times	1	1
Recaptured five times	1	0

DISCUSSION

Male anurans emit advertisement calls to attract females and announce their position to nearby males competing for the same territories and/or females (Wells 1977a). In many species, males can recognize the size and, consequently, the condition of their opponents evaluating the characteristics of the advertisement calls, which allows males to avoid fights they have low probabilities to win (Wells 1978, Robertson 1986, Bee and Perril 1996, Nali and Prado 2014). Thus, when body condition asymmetries between males are large and more easily assessed, less intense encounters are expected, because weaker individuals will probably give up earlier (Bee 2002). In our first experiment, the alpha males were larger than the betas, and the number of advertisement calls was positively correlated with male condition. This partially corroborates our first hypothesis, and suggests that males of *B. goiana* may be assessing the condition of their opponents through their calls, and thus avoiding costly conflicts with large males. However, a recent study on the acoustic behavior of *B. goiana* showed that males did not alter their vocal activity in response to playbacks simulating a larger opponent (Morais et al. 2016), suggesting that males may not be able to assess their opponents' size solely through their calls.

If *B. goiana* males do not assess the condition of their opponents via their calls to avoid unnecessary fights, what could explain the low prevalence of physical contact during contests? We suggest two non-exclusive explanations. The first relates to fighting motivation: if males had already mated before the experiments, this should decrease their motivation to engage in costly contest behaviors (Brown et al. 2007). In anurans, energetic depletion, and the resulting decrease in motivation, may occur before any measurable decrease in body condition (e.g. bullfrogs, Hettyey et al. 2009). Because we performed most of our experiments in the middle-late reproductive season (January-May), and given the constraints of in-situ experimental designs, if some males had previously mated, they are less likely to engage in

costly confrontations with other males. The second explanation deals with the low frequency of scars in males: very few males used in the experiments had scars, contrasting to species such as *Boana curupi* (Garcia, Faivovich & Haddad, 2007), in which 90% of the males exhibited fighting scars (Candaten et al. 2020). This could mean that either *B. goiana* is inherently less aggressive than other gladiator frogs or, alternatively, that resources were not limited enough to motivate aggression (Arnott and Elwood 2008). For example, males of *B. curupi* fight to monopolize a few oviposition sites along a stream, while oviposition in *B. goiana* occurred along the entire stream area (Dias et al. 2017, Candaten et al. 2020). However, to further test these hypotheses, comparisons among different populations and experiments controlling for previous matings are necessary. Nonetheless, it is important to highlight that the argument that *B. goiana* males do not evaluate their opponent's size via their calls comes from an experiment using playback (Morais et al. 2016). Thus, another possibility is that males of this species employ a multimodal communication, assessing their opponents using more than one type of signal, such as visual ones (Starnberger et al. 2014). Combination of acoustic and visual signals have been reported for several anuran species, including hylids closely related to *B. goiana*, such as *Boana albomarginata* (Spix, 1824) and *Boana albopunctata* (Spix, 1824) (Giasson and Haddad 2006, Toledo et al. 2007). These visual signals occur even in nocturnal species (Hartmann et al. 2004) and may be very subtle, such as opening the mouth or inflating the vocal sac without calling (Lipinski et al. 2012), behaviors that were observed in *B. goiana* during our experiments (T.M. Dias, pers. obs.). Thus, a possibility remains that physical combats were rare during our study because males were able to use more than one source of information to assess their opponents' size to avoid unnecessary conflicts, though this hypothesis needs further testing and does not exclude other possible explanations mentioned above.

The aggressive calls of *B. goiana* probably have the role of maintaining a spatial separation between calling males, and thus may serve as a warning to intruders (Wells 1977a, Dyson and Passmore 1992). The reduction of the acoustic interference, achieved through the maintenance of spacing between males, may be more important for male reproductive success than the possession of a particular calling site (Whitney and Krebs 1975, Wells 1977a, Nali and Prado 2014). Resident males are also more likely to win territorial disputes, because the cost of losing a territory is higher for a resident (Wells 1978, Bee et al. 1999, Owen and Gordon 2005). Additionally, large males usually win contests against small males (Howard 1978, Wells 1978). In our second experiment, large males emitted more aggressive calls and the more males in the chorus, the larger was the distance adopted by the nearest male. This suggests that males of *B. goiana* can detect the spatial position of other males and that intruder males can adjust their behavior according to the social context (Wells 1977b). Thus, maintaining a suitable distance from the resident male or avoiding areas where they are calling may be strategies adopted by intruder males of *B. goiana* to avoid undesired physical combats and/or acoustic interference. Similar behaviors were described for another species of gladiator frog, *Bokermannohyla ibitiguara* (Cardoso, 1983) (Nali and Prado 2012, 2014). Interestingly, the increase in male density did not lead to an increase in aggression levels or any other change in calling behaviors (Table 1), dismissing our second hypothesis. General theory predicts that competition should become more intense the more valuable (or scarce) a resource is (Grant 1993, Arnott and Elwood 2008). Since it does not seem to be the case for *B. goiana*, two explanations are possible. The first is a scenario where there are too many competitors, meaning that the costs of defense are high and any potential benefit does not pay off (Grant 1993). This does not seem to be the case, because even though aggression levels were low, there was a clear establishment and defense of territories, with only two males adopting alternative strategies such as satellite behavior. The second explanation could be that resource value does not increase enough to motivate an increase in aggressive behavior. This makes sense when we consider that, in our case, the resource is a mating opportunity. Although we predicted such opportunities are restricted across both time (the reproductive season) and space (the reproductive site), the results showed that this may not be the case – there is evidence that females of *B. goiana* mate more than once in the same reproductive season (discussed in more details below, and see Results). If mating opportunities are not as restricted as previously thought, investing in increased aggression may not be an adaptive strategy because such behavior is costly and risky (Briffa and Sneddon 2007), and maintaining the spacing between calling males in a chorus seems to be a more cost-effective strategy, as indicated by our results discussed above (Tables 1 and 2, Figs 2, 3).

Although most males were territorial, some males behaved as satellite males (sensu Wells 2007) during the territorial experiments and this strategy was already observed for many anuran species (e.g. Toledo and Haddad 2005, Leary et al. 2005, Nali and Prado 2012). This alternative strategy has been suggested to represent an attempt of males to intercept females approaching calling males or obtain a vacant territory (Wells 1977b, Haddad 1991). Another strategy would be engaging in a fight in order to obtain the resident's territory. However, physical combats were rare during both experiments and never exceeded 5 s (T.M. Dias, pers. obs.). Aggressive behaviors can include both aggressive calls and physical combats (Wogel et al. 2002, Costa et al. 2010 Lemes et al. 2012). However, as costs of physical combats can be high, males of *B. goiana* may be investing more in aggressive calls and, sometimes, adopting a satellite posture, which may be important to decrease energy expenditure and avoid injuries that could be caused by fights (Kluge 1981, Nali and Prado 2012). However, because satellite behavior only occurred in 2 of the 30 males used in the second experiment, the establishment of territories and maintenance of spacing between males mostly via calling behavior seems to be the norm for this population of *B. goiana*.

There was a positive relationship between the body size of males and females of *B. goiana* in amplexus, which indicates size-assortative mating and corroborates our third hypothesis. In some anuran species, females are able to assess male traits and choose those that might increase their fitness (Robertson 1990, Reynolds and Fitzpatrick 2007, Valencia-Aguilar et al. 2020). Size-assortative mating has been reported for some anurans and is suggested to favor a close juxtaposition of cloacae (Licht 1976, Morris 1989, Bastos and Haddad 1996, but see Green 2019). As most anurans exhibit external fertilization, better cloacae juxtaposition can maximize egg fertilization, potentially increasing the reproductive success of individuals in amplexus (Licht 1976, Robertson 1990, Bourne 1993, Bastos and Haddad 1996). In this context, it is likely that *B. goiana* females are choosing males with similar body sizes, though how females are evaluating the males (e.g. using acoustic signals, visual signals, or both) remains unanswered (see previous discussion regarding the males, and also Starnberger et al. 2014).

Most studies on anurans report a positive relationship between female size and clutch size/volume (e.g. Wogel et al. 2002, Dziminski and Alford 2005, Pupin et al. 2010, Guimarães et al. 2011). However, we did not find this relationship for *B. goiana*, and this same result was already observed for other hylid species (Lips 2001, Hartmann et al. 2004). We found that at least one female mated and spawned two times in the same reproductive season. In another occasion, after a female had spawned, we observed through the transparent skin of its abdomen that the female still carried several oocytes. These are evidences that *B. goiana* females may spawn more than once during each reproductive season, as observed for other anuran species (Rafínska 1991, Bastos and Haddad 1996, Vaira 2001). For species with multiple spawns, it has been suggested that the correlation between female size and size of clutches and eggs might be weak because clutches will differ in size between the beginning and the end of the breeding season (Parker and Begon 1986, Kusano and Hayashi 2002, Prado and Haddad 2005). Considering that egg size may represent a similar energetic cost for the female as the number of eggs per clutch, the same explanation could be applied for the absence of relationship between clutch size versus female size and between egg size versus eggs per clutch in this multiple-clutch species (Prado and Haddad 2005). Alternatively, the placement of the amplectant pairs in plastic bags to spawn may have affected their behavior. However, we think this is unlikely, because once ovulation begins the female is unable to prevent spawning. Thus, the observation of remaining oocytes in the abdomen of females and the lack of correlation between female size and clutch size/volume indicate that females of *B. goiana* spawn more than once during the reproductive season.

Our results differ in some aspects from what was previously known for *B. goiana*. For the population of *B. goiana* studied in Minas Gerais state, the mean number of eggs per clutch was higher and the mean egg size was smaller compared to our results (Menin et al. 2004). The average female SVL from the Minas Gerais population was larger than what we report here, which could explain the larger clutches found in that population (Menin et al. 2004). Furthermore, our sample size is larger and thus less likely to be affected by extreme values within the population that could affect the average values. Additionally, depending on the environmental conditions of the reproductive areas (e.g. competition, predation, food resources, unpredictability of water bodies), females may differ in the reproductive investment (e,g. fewer and larger eggs, many smaller eggs, eggs variable in size) (Crump 1984, Dziminski et al. 2009, Pupin et al. 2018). Considering the evidence for multiple spawning in *B. goiana*, this plastic response to dynamic environmental conditions seems plausible. Unfortunately, the available information does not allow us to infer the specific reasons for these reported differences.

We found that males were larger at the beginning of the reproductive season at our study site. Differences in the timing of male arrival may affect mating success, especially when the majority of the females arrive at the beginning of the reproductive season (Lodé et al. 2005, Wells 2007). Following this, large males arrive earlier than small males, although the reason for this is unclear (Salvador and Carrascal 1990, Lodé et al. 2005). One explanation could be that large males are older and more experienced, and thus know the reproductive area better and are able to arrive first (Reading 2001). However, this may not apply for *B. goiana* because no individual captured in the first season was recaptured in the second, suggesting that they may not live for more than one year. An alternative explanation could be that large males of *B. goiana* may simply have better physical conditions, arriving earlier at the area, and establishing territories first, as observed for *Rana dalmatina* Fitzinger, 1839 (Ranidae) (Lodé et al. 2005). A third explanation would be that the smaller size/condition of the males at the end of the reproductive season could reflect recruitment, as observed for *Boana albopunctata* (Spix, 1824) (de Sá et al. 2014). The reproductive season of *B. goiana* starts in October, and one possibility is that males born at the beginning of the season may already be sexually mature around May. If this is true, the entrance of these young, small males into the breeding active population may lower the average size of the males, thus explaining our results. Lastly, because resident males tend to lose weight throughout the reproductive season due to calling activity and territorial defense (Wells 1978, Robertson 1986), and may be dependent on sperm stored before the reproductive season (Hettyey et al. 2009), they may leave the chorus before the end of the breeding season. This could allow small males to enter the chorus and obtain a chance of reproducing in the late breeding season. An interesting technique that could help to discern between these explanations is to mark the tadpoles and keep track of them until maturity (e.g. Martin 2011, Courtois et al. 2013).

Males and females of *B. goiana* appear to be capable of adopting different reproductive strategies according to environmental conditions and social contexts. Females of this species much likely spawn multiple clutches, which can vary regarding

the number of eggs and egg size. It would be particularly interesting to follow females throughout an entire reproductive season, so that we could investigate trade-offs between egg size, egg number and female investment in different environmental conditions. Females might be obtaining information about calling males, and thus selecting those with similar body sizes, as evidenced by the size-assortative mating, which could increase their fitness via increased fertilization rates. Males of *B. goiana* seem to avoid fights in which individuals have low probabilities to win, because physical combats were rare. This is corroborated by the distance between calling males, which increased with the number of aggressive calls of the resident male. Alternatively, low aggression levels can be the result of low motivation or even an inherent trait of *B. goiana*, but these hypotheses require further investigation. The possibility that this species employs multimodal communication could also explain some inconsistencies with published data, although this also requires further evaluation. Acoustic communication seems to be extremely important for *B. goiana* in many social contexts and future studies should investigate which information, if any, are being assessed by individuals, as well as how this information could be conveyed.

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